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Peptide KED: Molecular-Genetic Aspects of Neurogenesis Regulation in Alzheimer's Disease

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Neuroprotective peptides are promising candidate molecules for the treatment of Alzheimer's disease (AD). Oral application of KED (Lys-Glu-Asp) improved memory and attention in elderly individuals with functional CNS disorders. Peptide KED also restores synaptic plasticity in *in vitro* model of AD. This review is focused on the analysis of the influence of KED peptide on the expression of genes and synthesis of proteins regulating apoptosis, aging, neurogenesis, and involved in AD pathogenesis. Analysis of published reports and our experimental findings suggests that KED regulates the expression of genes of cell aging and apoptosis (*p16*, *p21*), genes (*NES*, *GAP43*) and proteins (nestin, GAP43) of the neuronal differentiation, and genes involved in AD pathogenesis (*SUMO*, *APOE*, and *IGF1*). The study the effectiveness of neuroprotective peptide KED in animal models of AD seems to be very important.

Key Words: *peptide KED; gene expression; neuroprotection; Alzheimer's disease*

Currently, there are no sufficiently effective pharmacotherapy methods for Alzheimer's disease (AD) [9]. One of the treatment strategies is based on administration of drugs of peptide origin with physiological mechanism of action and high neuroprotective activity. Short peptides that are effective in *in vitro* and *in vivo* AD models appear to be promising. They have practically no side effects and do not cause allergic reactions. Some di-, tri- and tetrapeptides are practically not hydrolyzed in the blood and gastrointestinal tract and can be transported with Pept1, Pept2, Lat1, and Lat2 transporters into cells of various organs and tissues. Peptides activate signaling cascades in the cells by binding to target proteins or penetrate into the nucleus and interact with DNA and/or histone proteins, *i.e.* perform peptide regulation of gene expression and protein synthesis, the mechanism of which is unique for each peptide [4,6].

Peptide KED (Lys-Glu-Asp) was identified as a component of a polypeptide complex isolated from blood vessels [2]. Neuroprotective properties of this peptide after its oral administration to elderly individuals were revealed. KED peptide improved psychoemotional state, neurophysiological functions of the central nervous system and memory in patients with depression, apathy, memory and attention impairments [1]. In the primary culture of mouse hippocampal neurons in an AD model, KED peptide significantly increased the number of mushroom spines [3]. It is known that AD is associated with a decrease in the number of mushroom spines in hippocampal neurons involved in the mechanisms of neuroplasticity. Hence, KED peptide exhibited neuroprotective activity at the cellular (normalization of neuronal morphology) and organ (brain function) levels.

This review analyzes the results of the studies on the effects of KED peptide on gene expression and synthesis of proteins regulating neurogenesis and aging and involved in the pathogenesis AD.

EFFECT OF KED PEPTIDE ON NEURONAL DIFFERENTIATION OF HUMAN DENTAL STEM CELLS

Confocal microscopy and Western blot analysis showed that KED peptide 1.8-2.0-fold increased the

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synthesis of neurogenesis markers (nestin, GAP43) in human dental stem cells [7,12].

Nestin is expressed at the initial stage of neuronal differentiation and belongs to the cytoskeleton proteins [18,20,25,28]. Nestin is encoded by *NES* gene. Nestin expression is re-induced under pathological conditions, for instance, with glial scar that occurs after CNS injury [13]. It was found that nestin expression in pluripotent hippocampal cells decreased in mice with AD [26]. In mouse model of AD, memory normalization was observed under the influence of curcumin (γ-secretase inhibitor), which correlated with an increase in nestin expression in mouse hippocampus [14]. Transplantation of neuronal precursors expressing nestin contributed to a decrease in the manifestations of neurodegenerative changes in rats in the AD model [11].

GAP43 (growth associated protein 43) is expressed during neuronal differentiation and is involved in the generation and transmission of nerve impulses [19,27]. Increased concentrations of GAP43 protein in the cerebrospinal fluid were detected at the initial stage of AD. A correlation between the expression of Aβ42 peptide and GAP43 protein in the hippocampus, amygdala, and cerebral cortex was revealed in AD patients [20]. In another study, plasma concentration of GAP43 and APOE proteins were proposed as biomarkers of early AD [16]. Some substances that normalized memory in AD patients also stimulated the expression of GAP43 in the brain [9]. Stimulation of *GAP43* and *NES* gene expression and synthesis of GAP43 and nestin proteins involved in neurogenesis can decelerate the development of AD.

KED peptide stimulates neuronal differentiation of stem cells by regulating GAP43 and nestin expression. As these proteins are involved in the neurogenesis restoration and maintenance of the functional activity of brain neurons in AD, KED peptide can be assumed to contribute to the protective effect in patients with AD.

EFFECT OF KED PEPTIDE ON GENE EXPRESSION AND PROTEIN SYNTHESIS IN CELLULAR SENESCENCE

Real-time PCR and immunofluorescence confocal microscopy have shown that KED peptide 1.8-3.2-fold inhibits the expression of gerontogens *p16*, *p21* and the synthesis of the corresponding proteins in human dental stem cells in *in vitro* aging modeling [21].

Transcription factors 16, p21 inhibit activity of cyclin-dependent kinases, preventing phosphorylation of Rb retinoblastoma protein that regulates the cell cycle. It was found that expression of proteins p16 and p21 in neurons, liver cells, pancreas, spleen, skin and kidneys increases during aging and negatively correlates with life expectancy [23]. Expression of p16 and p21 proteins increased in astrocytes, glia, and neurons of the brain during aging. The authors suggest that p16 protein synthesis is activated to a greater extent during normal aging, while p21 expression is activated in age-associated diseases. It was found that during replicative aging of fibroblasts and epithelial cells, their ability for neuronal differentiation decreased, which correlated with the increase in p16 expression [22]. Gene expression and synthesis of p16 protein increased in neurons and astrocytes of the brain of mice in the AD model, which was accompanied by a decrease in cognitive functions [24]. It should be assumed that gero- and neuroprotectors will contribute to a decrease in gene expression and synthesis of p16, p21 proteins in cells and stimulate neuronal differentiation, as shown for KED peptide.

EFFECT OF KED PEPTIDE ON THE EXPRESSION OF GENES INVOLVED IN AD PATHOGENESIS

The expression of the *SUMO1* and *APOE* genes was found to decrease during replicative aging of human fetal bone marrow mesenchymal stem cells FetMSC. KED peptide stimulated the expression of *SUMO1* and *APOE* genes in "old" FetMSC by 1.2 and 2.2 times, respectively [5]. In addition, KED peptide increased the expression of IGF1 gene, which was reduced by 3 and 2 times during replicative and stationary aging of FetMSC, respectively [6].

SUMO1 (small ubiquitin-like modifier 1) is a protein involved in post-translational modifications of proteins that regulate nuclear transportation, transcription, apoptosis, cell cycle phases, and DNA damage repair. Violation of gene expression and SUMO1 protein synthesis leads to the loss of synaptic plasticity and memory decrease in animals [15]. It is assumed that the dysfunction of SUMO1 protein leads to the accumulation of vacuoles containing a toxic amyloid peptide Aβ42 in neurons [8]. The regulation of gene expression and SUMO1 synthesis by pharmacological agents is considered to be one of the promising approaches in the search for neuroprotectors effective in AD [17].

APOE is a blood plasma protein involved in cholesterol transportation, a product of *APOE* gene located in chromosome 19. In the brain, APOE is synthesized by astrocytes and microglia, participates in the transport of cholesterol and triglyceride metabolites, and is involved in the growth and restoration of neurons. In AD, APOE dysfunction can lead to hypercholesterolemia and accumulation of the neurotoxic peptide Aβ42 in the brain tissues [10].

IGF-1 is an insulin-like growth factor-1 that is involved in the endocrine, autocrine, and paracrine

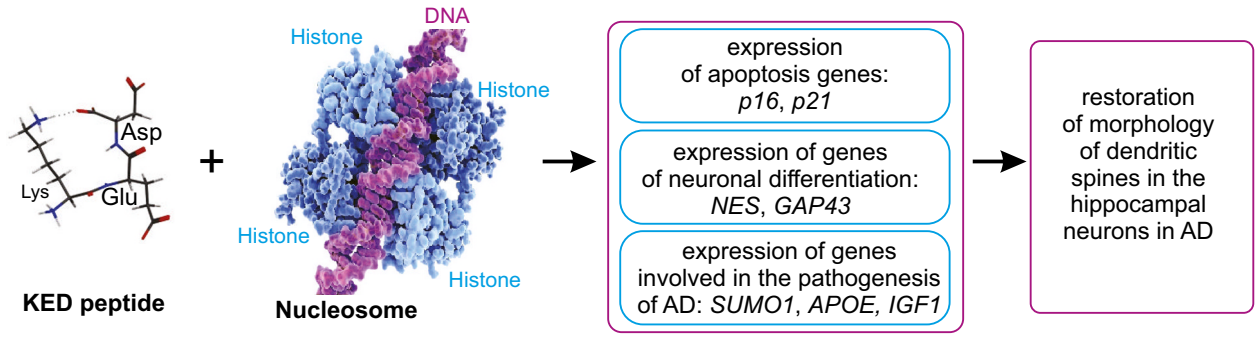

Fig. 1. Proposed scheme for the regulation of expression of neurogenesis-related gene by KED peptide. Possible role of KED peptide in neuroprotection in AD.

regulation of proliferation and differentiation of various types of cells, including neurons. IGF-1 was established to activate signaling cascades that prevent the accumulation of the toxic form of amyloid peptide Aβ42 and AD progression. Suppression of IGF-1 synthesis leads to the development of metabolic syndrome and AD. Exogenous administration of IGF-1 contributes to the normalization of synaptic plasticity in AD [28].

Thus, regulation of the *SUMO1*, *APOE*, and *IGF1* genes expression by KED peptide can help to prevent or slow down the development of pathological signaling cascades involved in the AD pathogenesis.

Analysis of the data presented suggests that KED peptide binds to the fragments of nucleosome (nucleobases of double-stranded DNA and/or histone proteins H1, H2b, H3, H4) and regulates the expression of genes responsible for cell aging and apoptosis (*p16, p21*), genes (*NES, GAP43*) and proteins (nestin, GAP43) of neuronal differentiation, genes and proteins involved in the AD pathogenesis (*SUMO1*, *APOE*, and *IGF1*) (Fig. 1). The decrease in the expression of proapoptotic genes *p16*, p21 and synthesis of corresponding proteins under the action of KED peptide decelerates death of hippocampal neurons. Activation of neuronal differentiation genes and synthesis of the corresponding proteins (nestin, GAP43) under the influence of KED peptide will help to maintain the pool of functionally active hippocampal neurons. Normalization of gene expression and synthesis of SUMO, APOE, IGF-1 proteins under the action of KED peptide, apparently, can prevent the development of pathogenetic cascades and reduce the synthesis of cytotoxic peptide Aβ42 and τ-protein. This, possibly, will contribute to the normalization and preservation of the dendritic spines of neurons and neuroplasticity maintenance, which is expressed in the preservation of memory and normalization of the central nervous system functions. Thus, KED peptide can be considered as a promising neuroprotector for studies in *in vivo* AD models.

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